Verhoeff–Van Gieson (VVG-NA) Stain

Laboratory Use Only

PRODUCT INFORMATION:

REF

SSP024 -NA 25 Reactions
SSP024- NA 50 Reactions

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Elastic Fibers: Black
Cell Nuclei: Blue-black
Collagen: Red
Other tissue elements: Yellow

SUMMARY AND EXPLANATION

For *laboratory* use only

The Verhoeff–Van Gieson (VVG-NA) Stain Kit is designed for the histological demonstration of elastic fibers, i.e., to identify their presence or absence in tissues. These fine elastic fibers are typically not visible on routine haematoxylin and eosin (H&E) stained tissue sections, and although they may appear refractile on H&E staining, they are often difficult to distinguish clearly from collagen fibers and smooth muscle. Consequently, the Verhoeff stain allows for the visualization of these delicate structures under standard light microscopy. This product is not intended for diagnostic or therapeutic purposes, and the results should be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

In the Verhoeff-Van Gieson (VVG) staining method, the tissue is stained with hematoxylin-ferric chloride and iodine containing working solution.

Elastic fibres have the strongest affinity for the hematoxylin-ferric chloride iodine solution which is used to overstain the entire tissue section. Therefore, the elastic fibres retain the dye, even after a diluted solution of ferric chloride is used for differentiation to break the tissue-mordant-dye complex in the other tissue elements. Sodium thiosulfate is used to remove excess iodine. The subsequent Van Gieson counter stain utilizes picric acid and acid fuchsin to stain collagen and muscle fibres, producing contrast against the hematoxylin stain. Under-differentiation is preferred in the Verhoeff stain, as picric acid used in the van Gieson counter stain serves to differentiate the elastic fibres further.

Kit Contents	Product Code	Storage Conditions	Pack Sizes	
			25 tests	50 tests
Hematoxylin - A (Reagent A)	IPS088A	RT	1.5g	2g
Ferric Chloride Solution - A (Reagent B)	IPS089	RT	7ml	15ml
Lugol's Iodine Solution (Reagent C)	IPS090	RT	7ml	15ml
Ferric Chloride Solution - B (Reagent D)	IPS091	RT	25ml	50ml
Sodium Thiosulphate Solution -A (Reagent E)	IPS051	RT	25ml	50ml
Vangieson Counter Stain Solution (Reagent F)	IPS092	RT	25ml	50ml

STORAGE AND HANDLING

Storage Recommendations: Store at Room Temperature. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after expiration date provided on the vial.**

To ensure proper regent performance delivery and stability, replace the dispenser cap after every use and immediately place the vials at recommended storage conditions away from sunlight in an upright position.

During transport, short-term exposure to 2-8°C does not affect product performance.

SPECIMEN PREPARATION

Sample Preparation and Fixation: Formalin-fixed, Paraffin-embedded tissue sections of 4- 5 µm thickness on microscopic slides

PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed.
2. This product should be used by qualified and trained professional users only
3. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
4. Dispose of waste observing all local, state, provincial or national regulations.
5. Do not use reagents after the expiration date
6. Use protective clothing and gloves while handling reagents
7. Avoid contamination of reagents, as it may lead to incorrect results

MATERIALS REQUIRED.BUT NOT PROVIDED:

- Xylenes
- Graded alcohols (50%, 70%, 95%, absolute)
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Cover slips
- Coplin jars
- Whatman Filter Paper
- Distilled water

PREPARATION OF WORKING SOLUTION

Refer to the pack size (listed on the box and empty labelled bottle) that is received before making any reagent preparations

Preparation of Hematoxylin solution – A:

Measure Absolute Alcohol (check pack size for measuring volume in the table below) into a beaker and then add Reagent A: Hematoxylin (check pack size for measuring volume in the table below). Mix thoroughly until the powder is completely dissolved or until the colour of the solution changes to a thick brown. Filter the solution by using Whatman Filter Paper into an empty labelled bottle (for storage) provided in the kit.

Components	Quantity Required	
Pack Size	25 Reactions	50 Reactions
Hematoxylin - A (Reagent A)	1.5g	2g
Absolute Alcohol (Not provided)	30ml	40ml

Verhoeff-Vangieson Working Solution:

Verhoeff-vangieson working solution should be made up fresh for the best results. Prepare the working solution by adding the following reagents in the similar order:

- Hematoxylin Solution - A : 0.5 ml
  (Refer to reagent preparation above)
- Ferric Choride Solution – A (Reagent B) : 0.25 ml
- Lugol's Iodine solution (Reagent C) : 0.25 ml

The above-mentioned volumes are sufficient for use on one slide. Mix the above amounts (or the required proportions thereof) thoroughly. Solution should be jet black. Use immediately and any leftover volume can be added to the slides during the incubation of this working solution.

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Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water—stain in verhoeff-vangieson working solution (Refer to the working solution preparation above) for 60 minutes. Tissue should be completely black.
2. Rinse in tap water with 2-3 changes and rinse in distilled water with 2-3 changes.
3. Differentiate in Ferric Chloride Solution - B (Reagent D) for 5-10 seconds with mild agitation.
   Note: As the time of differentiation is somewhat dependent on the amount of elastic tissue present, it is better not to rely on the control of timing for the differentiation of all sections, and slides must be individually differentiated to get good results, and also, it is better to rely on the side of under differentiation.
4. Stop differentiation with several changes of tap water and check microscopically for black elastic fiber staining and gray background. (It is better to slightly under differentiate the tissue, since the subsequent Van Gieson's counter stain can extract the elastic stain)
5. Wash slides in tap water.
6. Treat with Sodium Thiosulphate Solution - A (Reagent E) for 1 minute. Discard solution. Wash in running tap water for 3-5 minutes.
7. Counter stain in Vangieson Counter Stain Solution (Reagent F) for 2-3 minutes. (Counterstaining with Van Gieson's solution should not be prolonged, as picric acid present in this step differentiates the sections further)
8. Dehydrate quickly through 95% alcohol, 2 changes of 100% alcohol.
9. Clear in 3 changes of xylene for 2 minutes each.
10. Cover slip with Compatible mounting medium (DPX).

QUALITY CONTROL

The recommended positive tissue controls for Verhoeff–Van Gieson (VVG-NA) Stain Kit is Lung, Skin, Artery or any vascular tissue**.**

PERFORMANCE CHARACTERISTICS

The Verhoeff–Van Gieson (VVG-NA) Stain Kit stains the **Elastic fibres** in **black** color, **Cell Nuclei** in **Blue-black**, **Collagen** in **Red** and **other tissue elements** in **Yellow** color.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to data sheet provided.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results.
3. Do not allow the section to dry out during the entire staining process.
4. Gently mix all the reagents prior to use.
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.
11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. This product is intended for single-use application only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
14. PathnSitu makes no warranties beyond those expressly stated in the product description.
15. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
16. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
17. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

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2. *A histological study on the distribution of dermal collagen and elastic fibres in different regions of the body*; Naveen Kumar1, Pramod Kumar2, Keerthana Prasad and B. Satheesha Nayak1
3. *A modified Verhoeff-van Gieson elastin histochemical stain to enable pulmonary arterial hypertension model characterization* K.R. Percival, Z.A. Radi Pfizer Worldwide Research and Development, Drug Safety R&D, Andover, MA, USA

EXPLANATION OF SYMBOLS

LOT	Lot number / Batch number		Expiry
°C	Storage limitation	RT	Room Temperature
	Date of manufacture	REF	Catalogue number
	Manufacturer address		

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